

## Increased Mitochondrial $K_{ATP}$ Channel Activity During Chronic Myocardial Hypoxia

### Is Cardioprotection Mediated by Improved Bioenergetics?

Janis T. Eells, Michele M. Henry, Garrett J. Gross, John E. Baker

**Abstract**—Increased resistance to myocardial ischemia in chronically hypoxic immature rabbit hearts is associated with activation of ATP-sensitive  $K^+$  ( $K_{ATP}$ ) channels. We determined whether chronic hypoxia from birth alters the function of the mitochondrial  $K_{ATP}$  channel. The  $K_{ATP}$  channel opener bimakalim ( $1 \mu\text{mol/L}$ ) increased postischemic recovery of left ventricular developed pressure in isolated normoxic ( $\text{FiO}_2=0.21$ ) hearts to values ( $42\pm4\%$  to  $67\pm5\%$ ) not different from those of hypoxic controls but did not alter postischemic recovery of developed pressure in isolated chronically hypoxic ( $\text{FiO}_2=0.12$ ) hearts ( $69\pm5\%$  to  $72\pm5\%$ ). Conversely, the  $K_{ATP}$  channel blockers glibenclamide ( $1 \mu\text{mol/L}$ ) and 5-hydroxydecanoate (5-HD,  $300 \mu\text{mol/L}$ ) attenuated the cardioprotective effect of hypoxia but had no effect on postischemic recovery of function in normoxic hearts. ATP synthesis rates in hypoxic heart mitochondria ( $3.92\pm0.23 \mu\text{mol ATP} \cdot \text{min}^{-1} \cdot \text{mg mitochondrial protein}^{-1}$ ) were significantly greater than rates in normoxic hearts ( $2.95\pm0.08 \mu\text{mol ATP} \cdot \text{min}^{-1} \cdot \text{mg mitochondrial protein}^{-1}$ ). Bimakalim ( $1 \mu\text{mol/L}$ ) decreased the rate of ATP synthesis in normoxic heart mitochondria consistent with mitochondrial  $K_{ATP}$  channel activation and mitochondrial depolarization. The effect of bimakalim on ATP synthesis was antagonized by the  $K_{ATP}$  channel blockers glibenclamide ( $1 \mu\text{mol/L}$ ) and 5-HD ( $300 \mu\text{mol/L}$ ) in normoxic heart mitochondria, whereas glibenclamide and 5-HD alone had no effect. In hypoxic heart mitochondria, the rate of ATP synthesis was not affected by bimakalim but was attenuated by glibenclamide and 5-HD. We conclude that mitochondrial  $K_{ATP}$  channels are activated in chronically hypoxic rabbit hearts and implicate activation of this channel in the improved mitochondrial bioenergetics and cardioprotection observed. (*Circ Res.* 2000;87:915-921.)

**Key Words:** chronic hypoxia ■ 5-hydroxydecanoate ■ mitochondrial  $K_{ATP}$  channel

The ATP-sensitive  $K^+$  channel ( $K_{ATP}$  channel) is an important mediator of cellular protection in response to myocardial oxygen deprivation after chronic hypoxia and ischemia. Adaptation of hearts to chronic hypoxia results in enhanced activation of  $K_{ATP}$  channels.<sup>1</sup> Increased resistance to ischemia exhibited by chronically hypoxic rabbit hearts is associated with increased activation of the  $K_{ATP}$  channel.<sup>2</sup> Preconditioning in normoxic immature rabbit hearts is also associated with activation of the  $K_{ATP}$  channel.<sup>3</sup>

The precise cellular location at which the  $K_{ATP}$  channel mediates cardioprotection is unknown. If this can be identified, then the mechanisms through which  $K_{ATP}$  channels exert their protective effect may be determined. The cardioprotective effect of  $K_{ATP}$  channel openers, used at concentrations that do not shorten action potential duration, are abolished by the  $K_{ATP}$  channel blocker 5-hydroxydecanoate (5-HD).<sup>4</sup> Thus, 5-HD does not appear to act on the sarcolemmal  $K_{ATP}$  channel.  $K_{ATP}$  channels are also found in the inner mitochondrial membrane<sup>5,6</sup> where they control mitochondrial volume.<sup>7,8</sup> However, it is unknown if this  $K_{ATP}$  channel is

involved in mitochondrial energy production.<sup>9</sup> Diazoxide, a  $K_{ATP}$  channel opener, is 1000 times more selective for opening mitochondrial  $K_{ATP}$  channels than sarcolemmal channels.<sup>7</sup> The cardioprotective effect of diazoxide during ischemia is abolished by 5-HD, suggesting a role for the mitochondrial  $K_{ATP}$  channel in protection of the ischemic myocardium.<sup>10</sup> 5-HD abolished the cardioprotective effects of preconditioning in immature hearts, suggesting a cardioprotective role for mitochondrial  $K_{ATP}$  channels in immature hearts during conditions of oxygen deprivation.<sup>1</sup>

The present study further explores the involvement of mitochondria in the adaptation of heart muscle to chronic hypoxia. We hypothesize that activation of the mitochondrial  $K_{ATP}$  channel and its impact on mitochondrial bioenergetics may be an important event associated with increased resistance to ischemia in hearts adapted to chronic hypoxia. To assess the contribution of mitochondrial  $K_{ATP}$  channels, the rate of mitochondrial ATP synthesis was compared in normoxic and chronically hypoxic hearts. Our findings indicate that acute activation of the mitochondrial  $K_{ATP}$  channel

Received July 26, 2000; revision received September 19, 2000; accepted September 19, 2000.

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TABLE 1. Hemodynamic Values for Each Group

Groups	Before Drug			After Drug			Reperfusion (35 Minutes)			
	Heart Rate, bpm	Coronary Flow Rate, mL/min	LVEDP, mm Hg	Heart Rate, bpm	Coronary Flow Rate, mL/min	LVEDP, mm Hg	Heart Rate, bpm	Coronary Flow Rate, mL/min	LVEDP, mm Hg	Percent Recovery LVEDP
Normoxic, no intervention, control for bimakalim and glibenclamide	232±29	6±1	99±8	...	...	...	222±34	5±1	42±8†	42±4
Normoxic+bimakalim (1 $\mu$ mol/L)	231±19	6±1	99±6	270±16	12±1	96±5	228±17	5±2	66±7†	67±5
Normoxic+glibenclamide (1 $\mu$ mol/L)	229±18	6±1	98±6	168±21*	3±2*	52±8*	221±26	6±1	42±8†	43±5
Normoxic, no intervention, control for 5-HD	225±28	6±2	102±7	...	...	...	210±28	5±1	45±4†	44±4
Normoxic+5-HD (300 $\mu$ mol/L)	240±16	6±2	97±6	225±23	6±2	95±6	225±28	6±2	40±6†	41±4
Hypoxic, no intervention, control for bimakalim and glibenclamide	224±21	8±1‡	100±6	...	...	...	222±28	7±1	69±8†	69±5
Hypoxic+bimakalim (1 $\mu$ mol/L)	230±19	8±1‡	96±9	269±18	14±3	78±8*	219±16	5±2	69±10†	72±5
Hypoxic+glibenclamide (1 $\mu$ mol/L)	230±19	8±2‡	92±9	182±20*	4±2*	48±12*	206±31	7±2	40±9†	43±4
Hypoxic, no intervention, control for 5-HD	221±16	9±2‡	100±6	...	...	...	210±23	8±1	67±4†	67±5
Hypoxic+5-HD (300 $\mu$ mol/L)	236±11	10±2‡	102±6	210±23	9±2	103±7	210±23	7±2	53±5†	52±5

LVEDP indicates left ventricular developed pressure. Values are mean±SD from 6 hearts per group.

\* $P<0.05$  before drug vs after drug; † $P<0.05$  before drug vs reperfusion; and ‡ $P<0.05$  normoxic vs hypoxic.

increases  $K^+$  influx into mitochondria, resulting in a reduction in the driving force for ATP synthesis. In addition, these findings indicate that  $K_{ATP}$  channels are tonically active in mitochondria isolated from hypoxic hearts and that this tonic activity may play a role in the alteration of mitochondrial bioenergetics, which renders the hypoxic heart more resistant to myocardial ischemia.

## Materials and Methods

### Creation of Hypoxia From Birth

Pregnant New Zealand White rabbits were obtained from New Franken Research Rabbits (New Franken, Wis). Animals used in this study received humane care in compliance with the *Guide for the Care and Use of Laboratory Animals*, formulated by the National Research Council, 1996. For the hypoxic studies, the kits were born in a normoxic environment and then transferred to a hypoxic environment ( $P_{O_2}=0.12$ ) immediately after their first feeding.<sup>1-3</sup> The oxygen in the chamber was maintained at this level throughout the remainder of the study. For normoxic studies, the kits were raised under identical conditions except that  $P_{O_2}$  in the environmental chamber remained at 0.21 for the duration of the study. The age of the rabbits at the time of the study was 7 to 10 days.

### Assessment of Ventricular Function

The isolated rabbit heart model was used for these studies and was instrumented as previously described.<sup>2,11</sup> The standard perfusate used was Krebs-Henseleit bicarbonate buffer.<sup>12</sup> Immediately after aortic cannulation, hearts were perfused at a constant pressure of 43 mm Hg in the Langendorff mode for 30 minutes, during which time

balloons were placed in both the left and right ventricles. Biventricular function and coronary flow rate were then recorded under steady-state conditions.<sup>2,3</sup> Hearts were then perfused with either a  $K_{ATP}$  opener (bimakalim, 1  $\mu$ mol/L) or a  $K_{ATP}$  blocker (glibenclamide, 1  $\mu$ mol/L) or 5-HD, 300  $\mu$ mol/L for another 15 minutes before a 30-minute period of global, no-flow ischemia at 39°C. After the ischemic period, hearts were reperfused for 35 minutes, during which time the various indexes of cardiac function were again measured under steady-state conditions. Thus, each heart served as its own control.

### Mitochondrial ATP Synthesis, Membrane Potential, and Ventricular ATP Concentrations

Mitochondria were isolated from normoxic and hypoxic hearts by differential centrifugation as described by Solem and Wallace.<sup>13</sup> Cardiac mitochondria prepared by this methodology have been shown to be metabolically active with respiratory control ratios of 3.5 to 5.0 with succinate and 8.0 to 10.0 with glutamate/malate and corresponding ADP/O<sub>2</sub> ratios of 1.5 to 1.7 and 2.5 to 2.7. Mitochondrial ATP synthesis was measured in the presence of complex I substrates (pyruvate plus malate) as previously described.<sup>14</sup> Semi-quantitative measurements of the potential difference across the inner mitochondrial membrane were determined spectrofluorometrically using the dye rhodamine-123.<sup>15</sup> ATP concentrations were determined in ventricular tissue extracts by luciferin-luciferase lumimetry.<sup>16,17</sup>

### Statistical Analysis

Recovery of developed pressure was expressed as a percentage of its predrug value. A minimum of 6 hearts was used for each of the 10 conditions studied, and the results are expressed as mean±SD or

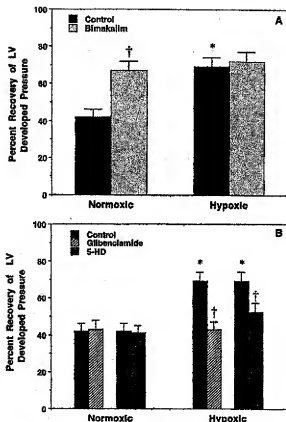


Figure 1. Effect of  $K_{ATP}$  channel openers and blockers on the postischemic recovery of ventricular function in normoxic and chronically hypoxic immature rabbit hearts. The  $K_{ATP}$  channel opener bimakalim ( $1 \mu\text{mol/L}$ ) (A) or the  $K_{ATP}$  channel blockers glibenclamide ( $1 \mu\text{mol/L}$ ) or 5-HD ( $300 \mu\text{mol/L}$ ) (B) were added 15 minutes before a global ischemic period of 30 minutes, followed by 35 minutes of reperfusion. Results are expressed as percent recovery of left ventricular pressure. Data shown are the mean  $\pm$  SE from 6 experiments. \* $P < 0.05$  normoxic vs hypoxic; † $P < 0.05$  control vs drug-treated. LV indicates left ventricular.

mean  $\pm$  SE. Statistical analysis was performed by use of repeated-measures ANOVA, with the Greenhouse-Geisser adjustment used to correct for the inflated risk of a type I error.<sup>18</sup> After ANOVA, the data were corrected for multiple comparisons. Significance was accepted at a level of  $P < 0.05$ .

## Results

**Contribution of the  $K_{ATP}$  Channel to Postischemic Recovery of Ventricular Function in Normoxic and Chronically Hypoxic Immature Rabbit Hearts**

Table 1 and Figure 1 illustrate the effects of bimakalim ( $1 \mu\text{mol/L}$ ), glibenclamide ( $1 \mu\text{mol/L}$ ), and 5-HD ( $300 \mu\text{mol/L}$ ) on the recovery of postischemic left ventricular function in hearts from normoxic and hypoxic rabbits perfused at constant pressure. These experiments were conducted using the same concentrations of  $K_{ATP}$  channel openers and blockers in the perfused hearts used to examine  $K_{ATP}$  function in isolated mitochondria. Recovery of postischemic left ventricular developed pressure in normoxic and hypoxic hearts was  $42 \pm 4\%$  ( $45 \pm 4\%$  for the normoxic no-intervention control for 5-HD) and  $69 \pm 5\%$  ( $67 \pm 4\%$  for the hypoxic no-intervention

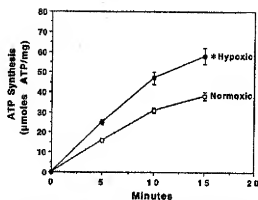


Figure 2. ATP synthesis in intact mitochondria isolated from hearts of normoxic and chronically hypoxic immature rabbits. ATP synthesis was measured in the presence of complex I substrates ( $1 \text{ mmol/L}$  pyruvate +  $1 \text{ mmol/L}$  malate) in mitochondria isolated from normoxic and hypoxic hearts. Results are expressed as  $\mu\text{mol ATP} \cdot \text{min}^{-1} \cdot \text{mg mitochondrial protein}^{-1}$ . Data shown are the mean  $\pm$  SE from 4 to 6 experiments. \* $P < 0.05$  normoxic vs hypoxic.

control for 5-HD), respectively, consistent with our previous findings showing that hypoxia increases the tolerance of the heart to subsequent ischemia.<sup>2</sup> As shown in Figure 1A, the  $K_{ATP}$  channel opener bimakalim ( $1 \mu\text{mol/L}$ ) increased recovery in normoxic hearts from  $42 \pm 4\%$  to  $67 \pm 5\%$  but had no effect on recovery of function in hypoxic hearts ( $72 \pm 5\%$ ). Thus, bimakalim increased the recovery of normoxic hearts to that observed in hypoxic hearts but did not alter subsequent recovery in hypoxic hearts. Conversely, the  $K_{ATP}$  channel blockers glibenclamide ( $1 \mu\text{mol/L}$ ) and 5-HD ( $300 \mu\text{mol/L}$ ) had no effect on recovery of developed pressure in normoxic hearts but decreased recovery in hypoxic hearts from  $69 \pm 5\%$  to  $43 \pm 4\%$  in experiments conducted with glibenclamide ( $1 \mu\text{mol/L}$ ) and from  $67 \pm 5\%$  to  $52 \pm 5\%$  in experiments conducted with 5-HD ( $300 \mu\text{mol/L}$ ) (Figure 1B).

## Effect of Chronic Hypoxia on Mitochondrial ATP Synthesis and Myocardial Energy Metabolism

ATP synthesis was measured in mitochondria isolated from hearts of normoxic and chronically hypoxic rabbits in the presence of the complex I substrates pyruvate and malate.<sup>14,15,17</sup> As shown in Figure 2, the rate of ATP synthesis in cardiac mitochondria was linear for 10 to 12 minutes in mitochondria isolated from both normoxic and hypoxic rabbits. The rate of ATP production in hypoxic heart mitochondria ( $3.82 \pm 0.23 \mu\text{mol ATP} \cdot \text{min}^{-1} \cdot \text{mg mitochondrial protein}^{-1}$ ) was significantly greater than the rate of ATP production in normoxic heart mitochondria ( $2.95 \pm 0.08 \mu\text{mol ATP} \cdot \text{min}^{-1} \cdot \text{mg mitochondrial protein}^{-1}$ ). ATP concentrations before the addition of respiratory substrates were  $63 \pm 4 \mu\text{mol/mg}$  of mitochondrial protein in normoxic heart mitochondria and  $73 \pm 12 \mu\text{mol/mg}$  in hypoxic heart mitochondria.

Other differences in myocardial energy metabolism were also apparent in chronically hypoxic versus normoxic immature rabbit hearts. Table 2 shows that ventricular lactate concentrations were twice as high in hypoxic hearts than normoxic hearts and ventricular lactate dehydrogenase

TABLE 2. Ventricular Energy Metabolites

	Normoxic ( $P_{iO_2}=0.21$ )	Hypoxic ( $P_{iO_2}=0.12$ )
Ventricular lactate, $\mu\text{mol/g dry wt}$	2 $\pm$ 1	4 $\pm$ 1†
Ventricular LDH, $\mu\text{IU/g wet wt}$	450 $\pm$ 51	608 $\pm$ 59†
Ventricular ATP, nmol ATP/mg tissue protein	9.83 $\pm$ 1.32	12.14 $\pm$ 1.42
Rate of mitochondrial ATP synthesis, $\mu\text{mol ATP} \cdot \text{min}^{-1} \cdot \text{mg mitochondrial protein}^{-1}$	2.95 $\pm$ 0.08	3.82 $\pm$ 0.23†

Values are mean $\pm$ SD from a minimum of 8 hearts in each group.

\*Data are from Baker et al.<sup>2</sup> 1997.

† $P<0.05$  normoxic vs hypoxic.

(LDH) concentrations were 35% greater in hypoxic than normoxic hearts. In addition, we have previously reported a shift in the LDH isoenzyme distribution toward the M or LD5 isoenzyme in hypoxic hearts.<sup>2</sup> These changes are indicative of an increased dependency on anaerobic glycolysis for energy production in hypoxic hearts. The combination of increased mitochondrial ATP production and increased glycolytic ATP production is likely to be responsible for the observation that myocardial ATP concentrations did not differ between normoxic and hypoxic hearts (Table 2).

#### $K_{ATP}$ Channel-Mediated Alterations in Mitochondrial ATP Synthesis

Activation of the mitochondrial  $K_{ATP}$  channel has been shown to increase  $K^+$  influx into the mitochondrial matrix, resulting in mitochondrial membrane depolarization and a reduction in the driving force for ATP synthesis.<sup>8,10</sup> The effects of  $K_{ATP}$  channel openers and blockers on ATP synthesis in mitochondria isolated from normoxic rabbit hearts are shown in Figures 3 and 4. As shown in Figure 3, the  $K_{ATP}$  channel opener bimakalim inhibited the rate of ATP synthesis in mitochondria isolated from normoxic rabbit hearts. In the presence of 1  $\mu\text{mol/L}$  bimakalim, the rate of ATP synthesis was reduced from  $2.96\pm0.10$   $\mu\text{mol ATP} \cdot \text{min}^{-1} \cdot \text{mg mitochondrial protein}^{-1}$  to  $1.56\pm0.22$   $\mu\text{mol ATP} \cdot \text{min}^{-1} \cdot \text{mg mitochondrial protein}^{-1}$ , a 52% reduction in the rate of ATP synthesis. The inhibitory action of bimakalim on mitochondrial ATP synthesis was sensitive to the  $K_{ATP}$  channel blocker glibenclamide (1  $\mu\text{mol/L}$ ). Glibenclamide (1  $\mu\text{mol/L}$ ) alone had no effect on the rate of ATP synthesis in normoxic heart mitochondria. However, the addition of glibenclamide (1  $\mu\text{mol/L}$ ) before the addition of bimakalim prevented the inhibition of ATP synthesis mediated by bimakalim. Figure 3 also shows that the reduction in ATP synthesis mediated by bimakalim (1  $\mu\text{mol/L}$ ) was abolished by the mitochondrial selective  $K_{ATP}$  blocker 5-HD (300  $\mu\text{mol/L}$ ). As with glibenclamide, 5-HD alone had no effect on the rate of mitochondrial ATP synthesis but prevented the reduction of ATP synthesis mediated by bimakalim.

Data presented in Figure 4 show that the mitochondria-specific  $K_{ATP}$  channel opener diazoxide (100  $\mu\text{mol/L}$ ) also reduced the rate of ATP synthesis from  $3.04\pm0.30$   $\mu\text{mol ATP} \cdot \text{min}^{-1} \cdot \text{mg mitochondrial protein}^{-1}$  to  $2.03\pm0.30$   $\mu\text{mol ATP} \cdot \text{min}^{-1} \cdot \text{mg mitochondrial protein}^{-1}$ , a 32% reduction in the rate of ATP synthesis. Furthermore, in nominally

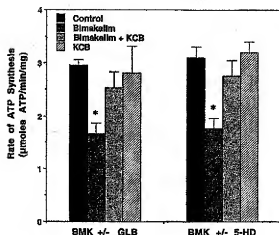
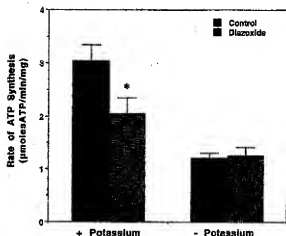


Figure 3. Effect of  $K_{ATP}$  channel openers and blockers on mitochondrial ATP synthesis in mitochondria isolated from normoxic immature rabbit hearts. Mitochondria isolated from normoxic hearts were incubated in the presence of vehicle (Control); bimakalim (1  $\mu\text{mol/L}$ ); bimakalim (1  $\mu\text{mol/L}$ )+glibenclamide (1  $\mu\text{mol/L}$ ); glibenclamide (1  $\mu\text{mol/L}$ ) alone; bimakalim (1  $\mu\text{mol/L}$ )+5-HD (300  $\mu\text{mol/L}$ ); or 5-HD (300  $\mu\text{mol/L}$ ) alone, and ATP synthesis was measured. Bimakalim inhibited ATP synthesis. The effect of bimakalim was antagonized by both glibenclamide and 5-HD, and glibenclamide or 5-HD alone had no effect on the rate of ATP synthesis. Results are expressed as  $\mu\text{mol ATP} \cdot \text{min}^{-1} \cdot \text{mg mitochondrial protein}^{-1}$ . Data shown are the mean $\pm$ SE from 4 to 6 experiments. \* $P<0.05$  control vs drug-treated. KCB indicates  $K_{ATP}$  channel blocker; GLB, glibenclamide; and BMK, bimakalim.

$K^+$ -free medium, diazoxide (100  $\mu\text{mol/L}$ ) had no effect on the rate of mitochondrial ATP synthesis indicating that the effect of  $K_{ATP}$  channel openers on mitochondrial ATP synthesis is dependent on the electrochemical gradient for  $K^+$ . The reduced rates of mitochondrial ATP synthesis measured in nominally  $K^+$ -free medium are likely due to an increase in  $K^+$ - $H^+$  antiport activity.<sup>7,8</sup> Although it is possible that a reduction in ATP synthesis might interfere with the action of  $K_{ATP}$  channel openers, the similarity of our findings with other studies demonstrating that the effects of  $K_{ATP}$  channel openers on mitochondrial membrane potential and mitochondrial swelling are dependent on the electrochemical gradient for  $K^+$  support this interpretation.<sup>8,9</sup>

#### Effects of $K_{ATP}$ Channel Openers and Blockers on ATP Synthesis in Mitochondria Isolated From Normoxic and Chronically Hypoxic Hearts

Figure 5A compares the effect of bimakalim on mitochondrial ATP synthesis in normoxic and hypoxic heart mitochondria. In mitochondria isolated from normoxic hearts, bimakalim produced a concentration-dependent decrease in the rate of ATP synthesis, reducing the rate of synthesis 50% at 1  $\mu\text{mol/L}$  and 60% at 10  $\mu\text{mol/L}$ . The rate of ATP synthesis in hypoxic heart mitochondria was not affected by the  $K_{ATP}$  channel opener bimakalim at concentrations of 1 or 10  $\mu\text{mol/L}$ . Figure 5B compares the effect of the  $K_{ATP}$  blockers glibenclamide (1  $\mu\text{mol/L}$ ) and 5-HD (300  $\mu\text{mol/L}$ ) on mitochondrial ATP synthesis in mitochondria isolated from normoxic and chronically hypoxic immature rabbit hearts. Nei-



**Figure 4.**  $K^+$  dependence of the effect of diazoxide on ATP synthesis in mitochondria isolated from normoxic immature rabbit hearts. ATP synthesis was measured in the presence of vehicle (Control) or diazoxide (100  $\mu$ M/L) in normoxic heart mitochondria incubated in buffer containing 110 mmol/L  $K^+$  and in nominally  $K^+$ -free solution (KCl replaced with 110 mmol/L choline chloride,  $K_2HPO_4$  replaced with 5 mmol/L  $Na_2HPO_4$ , and pH adjusted with trizma base). Diazoxide (100  $\mu$ M/L) produced a  $K^+$ -dependent inhibition of ATP synthesis in mitochondria isolated from normoxic hearts. Data shown are the mean  $\pm$  SE from 4 experiments. \* $P < 0.05$  control vs drug-treated.

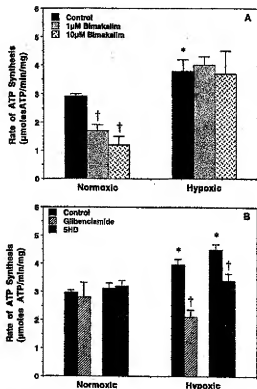
ther  $K_{ATP}$  blocker altered the rate of ATP synthesis in normoxic heart mitochondria; however, in hypoxic heart mitochondria, both glibenclamide and 5-HD significantly reduced the rate of ATP synthesis. Glibenclamide produced a 50% decrease in the rate of ATP synthesis and 5-HD reduced ATP synthesis by 25%.

#### Mitochondrial Membrane Potential in Mitochondria Isolated From Normoxic and Chronically Hypoxic Hearts

Semiquantitative measurements of mitochondrial membrane potential were determined using the fluorescent probe rhodamine-123.<sup>13</sup> In the absence of  $K_{ATP}$  channel modulators, resting membrane potential was remarkably similar in mitochondria isolated from normoxic and hypoxic hearts. Isolated cardiac mitochondria have been reported to have a membrane potential of  $-180 \pm 15$  mV in studies using the potential sensitive probe tetraphenylphosphonium.<sup>9</sup> Attempts to assess the effects of  $K_{ATP}$  channel openers or blockers in mitochondria isolated from normoxic and hypoxic hearts using rhodamine-123 were confounded by interactions between the vehicle or the drugs and the fluorescent probe.

#### Discussion

We have demonstrated in rabbits that chronic exposure to hypoxia from birth increases the resistance of the heart to subsequent ischemia<sup>12,13</sup> and that glibenclamide, a  $K_{ATP}$  channel blocker, abolishes this cardioprotective effect. More recently, we have shown that ischemic preconditioning in immature rabbit hearts also increased resistance to ischemia and that 5-HD abolished this cardioprotective effect.<sup>3</sup> Thus,



**Figure 5.** Effect of  $K_{ATP}$  channel openers and blockers on ATP synthesis in mitochondria isolated from normoxic and chronically hypoxic immature rabbit hearts. A, ATP synthesis was measured in normoxic heart mitochondria incubated in the presence of vehicle (Control) or bimakalim at concentrations 1 or 10  $\mu$ M/L. Bimakalim produced a concentration-dependent inhibition of ATP synthesis in mitochondria isolated from normoxic hearts; however, bimakalim had no effect on the rate of ATP synthesis in mitochondria isolated from chronically hypoxic hearts. B, ATP synthesis was measured in normoxic or hypoxic heart mitochondria treated with glibenclamide (1  $\mu$ M/L) or 5-HD (300  $\mu$ M/L). Glibenclamide and 5-HD had no effect on the rate of ATP synthesis in normoxic heart mitochondria. In contrast, both  $K_{ATP}$  channel blockers inhibited the rate of ATP synthesis in hypoxic heart mitochondria. Data shown are the mean  $\pm$  SE from 4 experiments. \* $P < 0.05$ , normoxic vs hypoxic; † $P < 0.05$  control vs drug-treated.

ischemic preconditioning and adaptation to chronic hypoxia in immature hearts appear to share a final common effector, the  $K_{ATP}$  channel.

In light of recent studies implicating the mitochondrial  $K_{ATP}$  channel in cardioprotection,<sup>18,19</sup> we conducted experiments to examine the role of the mitochondrial  $K_{ATP}$  channel in adaptation to chronic hypoxia in immature hearts. To assess mitochondrial  $K_{ATP}$  channel function, we measured the effect of several  $K_{ATP}$  channel openers and blockers on mitochondrial ATP synthesis in metabolically active mitochondria isolated from hearts of normoxic and chronically hypoxic rabbits. This approach was predicated on the knowledge that activation of the mitochondrial  $K_{ATP}$  channel has been shown to increase the influx of  $K^+$  into mitochondria, resulting in mitochondrial depolarization and a reduction in the rate of ATP synthesis.<sup>2</sup> The actions of  $K_{ATP}$  channel openers and blockers in mitochondria isolated from

normoxic and hypoxic hearts paralleled their actions on cardiac function in isolated perfused hearts.  $K_{ATP}$  channel activation by bimakalim resulted in a decrease in the rate of ATP synthesis in normoxic heart mitochondria but had no effect on ATP synthesis in hypoxic heart mitochondria. Similarly,  $K_{ATP}$  channel activation markedly enhanced recovery of ventricular function in normoxic hearts but had no effect on functional recovery in hypoxic hearts. In normoxic heart mitochondria, the  $K_{ATP}$  blockers glibenclamide and 5-HD had no effect on the rate of ATP synthesis, suggesting that mitochondrial  $K_{ATP}$  channels are not tonically active. These blockers also had no effect on recovery of function in normoxic hearts. In contrast, in hypoxic heart mitochondria,  $K_{ATP}$  channel blockers reduced the rates of ATP synthesis to rates similar to those observed in normoxic heart mitochondria. In hypoxic hearts, both  $K_{ATP}$  blockers significantly attenuated cardioprotection. These results corroborate our previous findings in isolated perfused hearts<sup>1,3</sup> and strongly suggest that enhanced activation of the mitochondrial  $K_{ATP}$  channel is an important component of the cardioprotective mechanisms involved in adaptation to hypoxic stress.

A second significant finding of these studies was the increased rate of ATP synthesis observed in mitochondria isolated from chronically hypoxic hearts. Moreover, there was no difference in myocardial ATP concentrations or in mitochondrial membrane potential in hypoxic versus normoxic hearts. One potential explanation for the apparent discrepancy between the inhibition of the rate of ATP synthesis observed in mitochondria isolated from normoxic immature rabbit hearts versus the enhanced rate of ATP synthesis after chronic hypoxia may be due to differences between acute versus chronic activation of the mitochondrial  $K_{ATP}$  channel. In the acute situation (i.e., mitochondria isolated from normoxic hearts), activation of the mitochondrial  $K_{ATP}$  channel by  $K_{ATP}$  channel openers results in  $K^+$  influx into mitochondria, mitochondrial depolarization, and a reduction in the driving force for ATP production measured in the present studies as a reduction in the rate of ATP synthesis. Our data further indicate that chronic hypoxia produces a tonic activation of the mitochondrial  $K_{ATP}$  channel. This is likely to result in adaptive changes in mitochondrial physiology. The observation that resting mitochondrial membrane potential did not differ between mitochondria isolated from normoxic or hypoxic hearts provides further evidence of an adaptive response to tonic activation of the mitochondrial  $K_{ATP}$  channel. Other studies have provided evidence that mitochondrial bioenergetics and metabolism are fundamentally altered by chronic hypoxia with changes reported in mitochondrial creatine kinase activity and in the ADP and  $O_2$  dependence of mitochondrial respiration.<sup>24,26</sup> Our findings suggest that an alteration in mitochondrial  $K_{ATP}$  channel function may be another component in mitochondrial adaptation to hypoxia. Recent studies showing involvement of the mitochondrial  $K_{ATP}$  channel in adaptation to high-altitude hypoxia further support this interpretation.<sup>27</sup>

Taken together, our findings suggest that the cardioprotective effects of mitochondrial  $K_{ATP}$  channel activation may be linked to improved oxidative metabolism and mitochondrial bioenergetics. An important role of the mitochondrial  $K_{ATP}$  channel is to regulate mitochondrial volume, which in turn is thought to

regulate electron transport and bioenergetics.<sup>5,3-40</sup> Opening of the mitochondrial  $K_{ATP}$  channel has been shown to shift the balance between  $K^+$  uniport and  $K^+-H^+$  antiport, resulting in transient net  $K^+$  uptake and increased matrix volume.<sup>5,6</sup> Halestrap<sup>7</sup> has established that small increases in matrix volume stimulate electron transport and that activation of the mitochondrial  $K_{ATP}$  channel may trigger this response. Mitochondrial  $K_{ATP}$  channel activation may therefore be an essential component of a signal transduction pathway calling for increased ATP production to support increased work in the heart or possibly to compensate for decreased oxygen availability. Conversely, blockade of the mitochondrial  $K_{ATP}$  channel may interfere with the cellular or mitochondrial response to these signals. The reduction in the rate of ATP synthesis observed in mitochondria from hypoxic hearts treated with  $K_{ATP}$  channel blockers is consistent with this interpretation.

We have suggested that adaptation to chronic hypoxia represents a unique form of preconditioning, and we have recently supported this contention by showing that although immature normoxic hearts can be preconditioned, immature hypoxic hearts cannot be preconditioned.<sup>7</sup> Furthermore, we have shown that the mechanism of preconditioning in the immature normoxic heart is associated with  $K_{ATP}$  channel activation and is abolished by the mitochondrial  $K_{ATP}$  channel blocker 5-HD.<sup>12</sup> Although a direct link between mitochondrial  $K_{ATP}$  channel activation and myocardial protection remains to be established, several known consequences of mitochondrial  $K_{ATP}$  channel activation are likely to improve mitochondrial function after ischemia. Activation of the mitochondrial  $K_{ATP}$  channel results in  $K^+$  influx into mitochondria, expansion of mitochondrial matrix volume, and a reduction of the inner mitochondrial membrane potential established by the proton pump.<sup>10</sup> Regulation of matrix volume is an essential element in the regulation of mitochondrial energy production, and matrix expansion secondary to mitochondrial  $K_{ATP}$  channel opening has been postulated to activate electron transport and stimulate mitochondrial metabolism.<sup>7</sup> Our findings of increased rates of ATP synthesis in mitochondria isolated from hypoxic hearts are consistent with this mechanism.

In summary, our data in conjunction with the studies of other investigators support a role for mitochondrial  $K_{ATP}$  channel activation and its impact on mitochondrial bioenergetics as an important factor in increased resistance to ischemia in hearts adapted to chronic hypoxia.

### Acknowledgments

This work was supported by grants from National Heart, Lung, and Blood Institute (HL-08311 and HL-45048) and from the National Institute of Environmental Health Sciences (ES-06648). The authors wish to thank Patricia Holman for her excellent technical assistance.

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